

09/993,314 filed 11/05/2001
Reply to Office Action of December 19, 2005

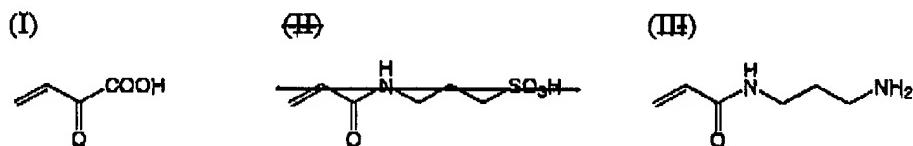
Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (currently amended) A method of performing a mobility shift assay in a microfluidic device, the method comprising:

flowing a reaction mixture comprising an enzyme, an enzyme substrate, and a product through a separation region of the microfluidic device under an applied pressure, which separation region comprises at least one ion-exchange material comprising a polyacrylamide material or a dimethylacrylamide material modified by one or more additives having formula (I), or (II), or (III):



to separate the product from at least one other material based upon a net charge difference between the product and the at least one other material to produce separated materials; and,

detecting at least one of the separated materials, thereby performing the mobility shift assay in the microfluidic device.

2. (original) The method of claim 1, wherein the at least one other material comprises the enzyme and/or unreacted enzyme substrate.

3. (original) The method of claim 1, wherein at least the separated materials are flowed in the microfluidic device in an absence of an applied electric field.

4. (original) The method of claim 1, wherein at least the separated materials are flowed in the microfluidic device under at least one simultaneously applied electric field.

5. (original) The method of claim 1, wherein one or more of the separated materials comprise a label.

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6. (original) The method of claim 1, wherein a microchannel comprises the separation region.

7. (original) The method of claim 1, wherein the applied pressure is produced by a vacuum pump operably connected to the microfluidic device through a port that fluidly communicates with the separation region.

8. (original) The method of claim 1, wherein the detecting step comprises at least an optical, a spectroscopic, a fluorescent, a mass, or a luminescent detection.

9. (original) The method of claim 1, wherein a plurality of microbeads or a gel comprises the ion-exchange material.

10. (original) The method of claim 1, wherein an inner surface of the separation region comprises the ion-exchange material.

11. (original) The method of claim 1, wherein the ion-exchange material is coated on an inner surface of the separation region.

12. (original) The method of claim 1, wherein the ion-exchange material comprises one or more of: a polyarginine, a polylysine, a modified polyacrylamide, or a modified dimethylacrylamide.

13. (canceled)

14. (original) The method of claim 1, further comprising sampling the reaction mixture from a source external to the microfluidic device.

15. (original) The method of claim 1, wherein the enzyme comprises a kinase, the enzyme substrate comprises a kinase substrate, and the product comprises a phosphorylated product.

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16. (original) The method of claim 15, wherein the kinase comprises a protein kinase, a protein kinase A, a protein kinase B, a protein kinase C, a hexokinase, a phosphofructokinase, a phosphoglycerate kinase, a pyruvate kinase, a cyclic AMP-dependent protein kinase, a cyclic GMP-dependent protein kinase, a calmodulin-dependent protein kinase II, a casein kinase I, a casein kinase II, a glycogen synthase kinase-3, a cyclin-dependent kinase, a p34/cdc2 kinase, or a nucleic acid kinase.

17. (original) The method of claim 1, wherein the enzyme comprises a phosphatase, the enzyme substrate comprises a phosphatase substrate, and the product comprises a dephosphorylated product.

18. (original) The method of claim 17, wherein the phosphatase comprises a protein phosphatase, an acid phosphatase, an alkaline phosphatase, a sugar phosphatase, or a polynucleotide phosphatase.

19. (original) The method of claim 1, wherein prior to the flowing step, the method comprises:

flowing at least the enzyme through a first channel in fluid communication with an enzyme source into a mixing region of the microfluidic device; and,

flowing at least the enzyme substrate through a second channel in fluid communication with an enzyme substrate source into the mixing region, wherein the enzyme converts at least some of the enzyme substrate to the product, thereby producing the reaction mixture.

20. (original) The method of claim 19, wherein a microchannel comprises the mixing region.

21. (original) The method of claim 1, the method further comprising flowing the ion-exchange material into the separation region.

22. (original) The method of claim 21, wherein the flowed ion-exchange material coats an inner surface of the separation region.

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23. (original) The method of claim 21, comprising continuously flowing the ion-exchange material into the separation region for a selected period of time.

24. (original) The method of claim 21, comprising flowing multiple aliquots of the ion-exchange material into the separation region.

25. (original) The method of claim 21, wherein the ion-exchange material is stored in a reservoir, which reservoir is in fluid communication with the separation region.

26. (previously presented) The method of claim 21, further comprising flowing one or more other chromatographic materials or surface coatings into the separation region.

27. (previously presented) The method of claim 26, further comprising flowing the ion-exchange material and the other chromatographic materials or surface coatings sequentially into the separation region.

28. (original) The method of claim 27, wherein each sequentially flowed material or surface coating coats or modifies an inner surface of the separation region or a previously flowed material which coats the inner surface of the separation region.

29. (original) The method of claim 1, the flowing step further comprising flowing one or more eluents or separation buffers into the separation region from one or more microchannels in fluid communication with the separation region.

30. (original) The method of claim 29, further comprising varying a concentration of the one or more eluents or separation buffers flowed into the separation region to control separation of materials within the separation region.

31. (original) The method of claim 1, further comprising sampling the enzyme, the enzyme substrate, and/or an additional material from one or more sources external to the microfluidic device.

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32. (original) The method of claim 31, wherein the additional material comprises one or more of: a modulator, an inhibitor, an antagonist, an agonist, an eluent, or a separation buffer.

33. (original) The method of claim 31, wherein the one or more sources are present in a microtiter dish and wherein the microfluidic device comprises one or more external capillary elements in fluid communication with the separation region, the method comprising contacting the one or more external capillary elements to the one or more sources and drawing fluid out of the one or more sources, into the one or more external capillary elements, and into the microfluidic device.

34-62 (canceled)

63 (canceled)